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Temperature and Voltage Dependence of Lipid Membrane Capacitance and the Corresponding Capacitive Currents
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Lipid bilayers and monolayers display a voltage dependent chain-melting transition behavior. Such melting transitions also exist in biomembranes close to physiological temperature, and therefore voltage induced transitions may be of biological relevance. Since changes in lipid state involve changes in membrane thickness, one obtains the possibility of a voltage-induced change in capacitance. This phenomenon leads to a capacitance maximum in the melting transition of membranes similar to heat capacity maxima. It further implies, that the often made assumption of constant capacitance in biomembranes (as made in most electrophysiological models) is incorrect close to transitions. As a striking consequence, this finding leads to the possibility of capacitive currents against the applied field with a time-scale of several milliseconds or more, which is the relaxation time of the lipid state after a perturbation by voltage. Simultaneously, the accompanying membrane permeability changes resulting in an ion current along the applied field.

Platform: Micro & Nanotechnology: Nanopores I

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Parallel Acquisition of High Resolution Polymer Mass-Spectra on a Nanopore Microbilayer Array
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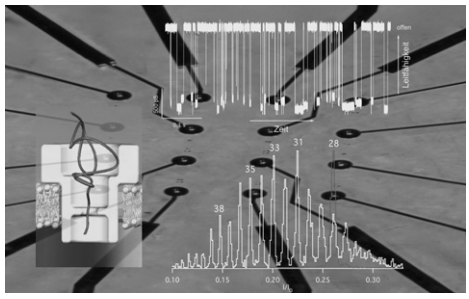
A more widespread use of protein nanopores with their many attractive applications in chemical and biological analytics will depend on the availability of bilayer recording methods that enable rapid collection of large amounts of data and can be easily automated. Nanopore-based single-molecule analysis typically require require low noise and high frequency bandwidth recordings so that low-capacitance microbilayers on smaller-than-standard (e.g. < 100 μm) bilayers are required.
We have formed lipid bilayers of <20 μm diameter containing single alpha-hemolysin (aHL) pores on arrays of sub-picoliter cavities containing individual microelectrodes (microelectrode cavity array, MECA) and ion conductance-based single molecule mass spectra of polydisperse mixtures of poly(ethylene glycol) molecules were simultaneously obtained from multiple single aHL-recordings. We thereby demonstrate the function of the MECA device as a chip-based platform for array-format nanopore recordings with a resolution at least equal to that of established single microbilayer supports. We conclude that devices based on MECAs may enable more widespread analytical use of nanopores by providing the high throughput and ease of operation of a high-density array format while maintaining or exceeding the precision of state-of-the-art microbilayer recordings.



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Layer-By-Layer Assembly of Cellular Structures
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Reconstituted systems have played an integral part in understanding biological structure and function in, for example, complex biosynthetic and signal transduction pathways. The road to similar model systems that recapitulate complex membrane-based functions, such as division or endocytosis, has been much more difficult due to the lack of synthetic routes to well-defined membrane bilayers. We have developed layer-by-layer membrane assembly, which enables the construction of arbitrarily complex - and thus far synthetically inaccessible - membranous structures, from asymmetric phospholipid bilayers to the double bilayers of mitochondria and the nuclear envelope. Starting with trapped lipid-stabilized water-in-oil droplets, a new lipid monolayer is deposited on the droplet, completing a bilayer templated on the starting droplet when a water/oil interface is driven over the droplet. Individual phospholipid monolayers are sequentially deposited each time a phase boundary passes over a trapped droplet, enabling the controlled construction of multi-lamellar membranes. Membrane compositional asymmetry was demonstrated by selectively depositing dye-labeled dioleoylphosphoethanolamine in either the inner or the outer leaflet of the bilayer followed by probing with a reductive quencher. Double bilayer membranes were also assembled to demonstrate control over membrane lamellarity. Dye-labeled phospholipids were selectively deposited in a single monolayer or simultaneously in multiple of the four constitutent monolayers and again probed with reductive quencher to confirm their location within the multi-lamellar membranes. The product vesicles can serve as both compartments and scaffolds for sophisticated cell-like metabolic activity (e.g. RNA transcription, protein expression, integral membrane protein insertion and membrane protein-mediated transport). These biomimetic vesicles of defined size form the core of an extensible and systematic platform for probing interactions between membrane proteins, lipid bilayers and chemical control of bilayer structure and remodeling events.

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Formation of Biomimetic Membrane Rafts on Bare and Modified Gold
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Lipid domains are essential functional entities of biological membranes, therefore lipid bilayers containing liquid disordered/liquid ordered (lipid rafts) phase coexistence are improved biomimetic systems. Moreover, supported lipid bilayers (SLB) of such ternary lipid mixtures formed on gold are promising interfaces for biosensing purposes. However, gold's hydrophobic nature has been an obstacle for direct deposition, and most studies rely on previous modification of its surface. In addition, the formation and properties of lipid domains in these systems only raised attention very recently. In this work, ternary lipid mixtures (e.g. DOPC/DPPC/Cholesterol (2:2:1 molar ratio)) were deposited on gold, either bare or modified with hydrophilic self-assembled monolayers (SAMs) of 11-mercaptoundecanoic-acid (MUA) and cystein, which display distinct packing density and organization. Atomic force microscopy was used to study the topography of the lipid films at the nanoscale, allowing the first reported observation of well-organized raft-containing SLB on unmodified gold. This was achieved under optimized conditions, as changing slightly buffer composition may lead e.g. to the formation of tubular structures. Coverage and continuity of SLB were addressed by ellipsometry and cyclic voltammetry, taking advantage of gold optical/electrical properties. The cyclic voltammograms of ferricyanide redox process suggest the presence of continuous bilayers with small pores. The bilayer thicknesses, 4-5nm, estimated by ellipsometry were further confirmed by force spectroscopy. The raft-containing bilayer is stable over a wide range of potential sweep, enabling the development of new lipid raft-based biosensing interfaces. Planar and continuous SLB could be also prepared onto gold modified electrodes. In the case of a short cystein SAM, pronounced decrease of the redox process intensity was observed after lipid deposition revealing a high coverage of the modified gold surface.
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Separating and Sorting Membrane Species using a Supported Bilayer Extractor Composed of Patterned Lipid Phases
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The role of lipid rafts is important in understanding cell processes and disease states; however, identifying the key components of these lipid domains is difficult and impedes research progress. Here we present a new platform to separate, sort, and characterize membrane-bound molecules based on their affinity for raft phase domains using a heterogeneous supported lipid bilayer (SLB) consisting of patterned lipid raft and non-raft domains. Adjacent SLBs consisting of liquid-ordered and liquid-disordered phases are formed inside a microfluidic channel and maintain phase separation. Membrane-bound biomolecules loaded into the device are convected laterally in the two-dimensional plane along the heterogeneous supported bilayer by a shear force induced from hydrodynamic flow of the bulk aqueous phase. During axial convection, the membrane-bound biomolecules diffuse across the microchannel within the 2-D heterogeneous bilayer plane, partitioning into their preferred lipid phase. Patterns for the lipid phases are designed to facilitate the sorting and collection of separated species. The main advantages of this new method over existing methods used to identify and assay raft species include separating membrane species within a membrane environment, near physiological conditions, and without artifacts associated with detergent, high salt, or alkaline pH. These criteria are particularly crucial to the separation of lipid-linked proteins and